

FIG. 1A

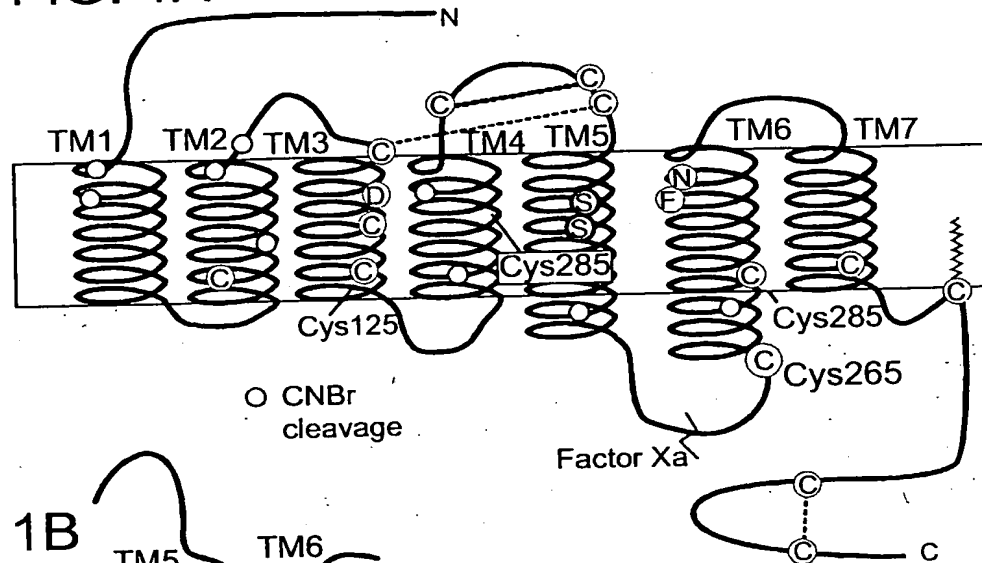
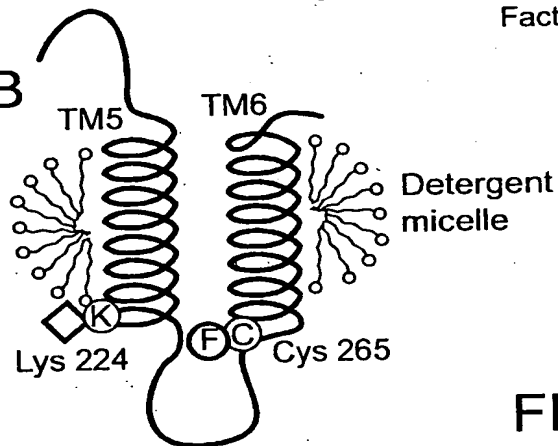
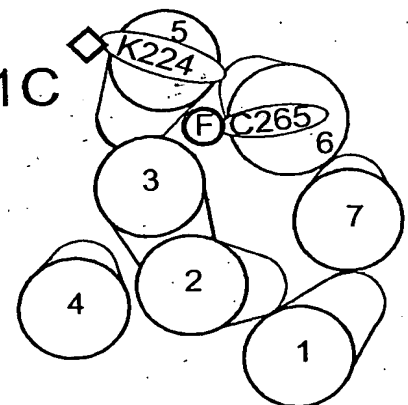


FIG. 1B



ⓕ FLUORESCIN
MALEIMIDE
◇ OXYL-NHS
(quencher)

FIG. 1C



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FIG. 2A

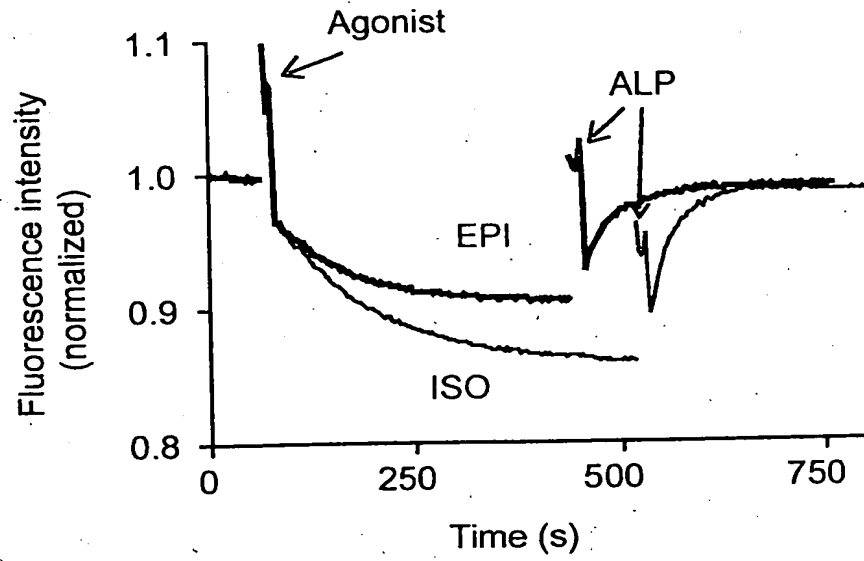
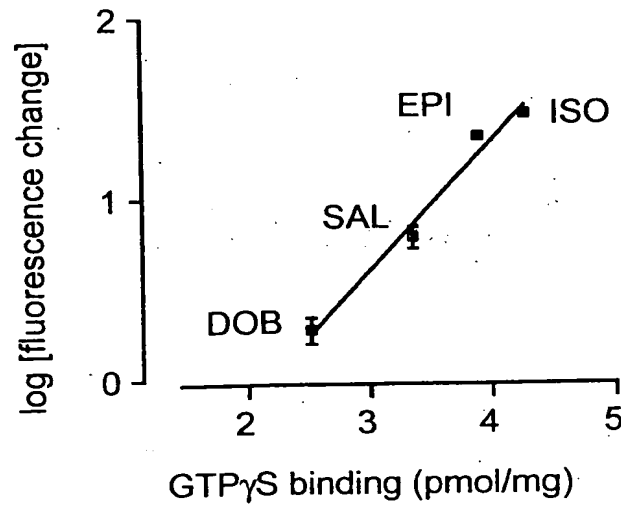


FIG. 2B



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FIG. 3A

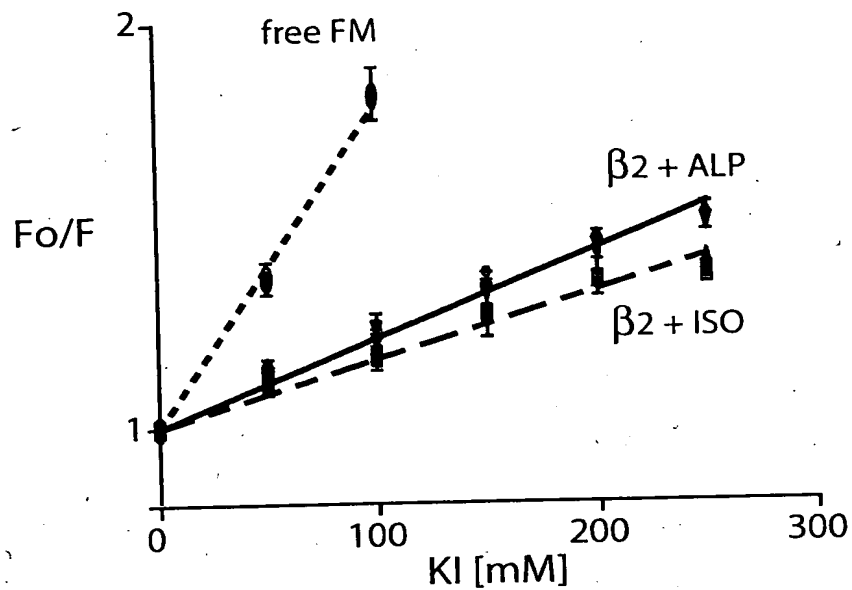
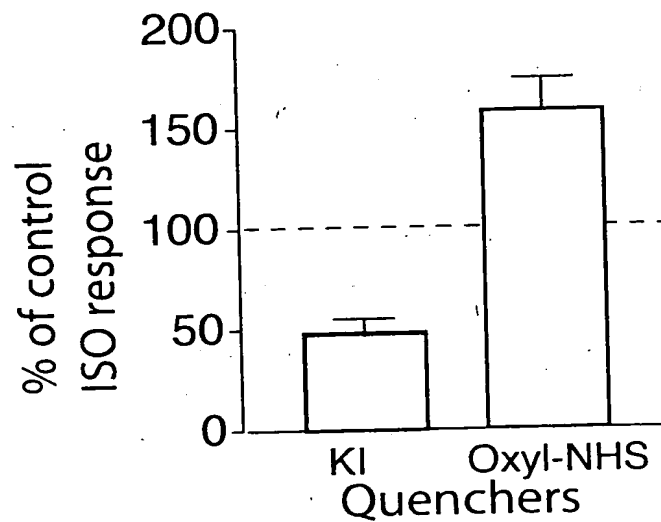


FIG. 3B



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FIG. 4A

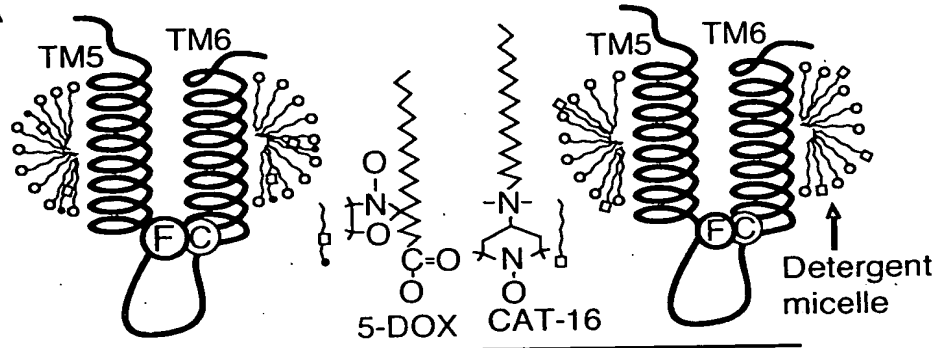


FIG. 4B

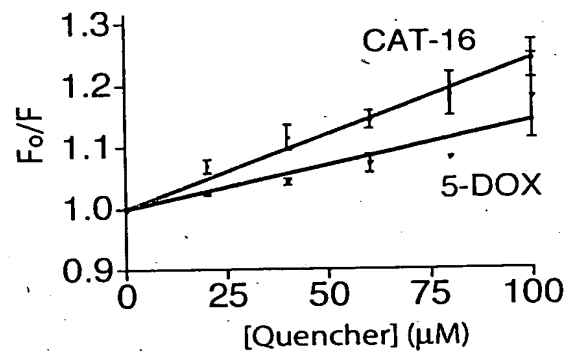


FIG. 4C

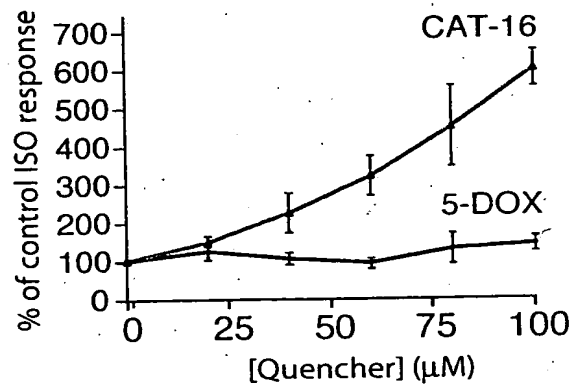
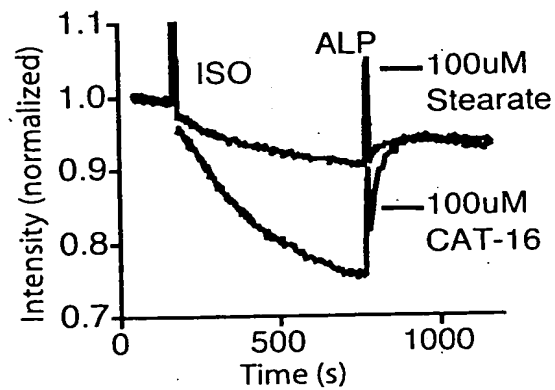


FIG. 4D



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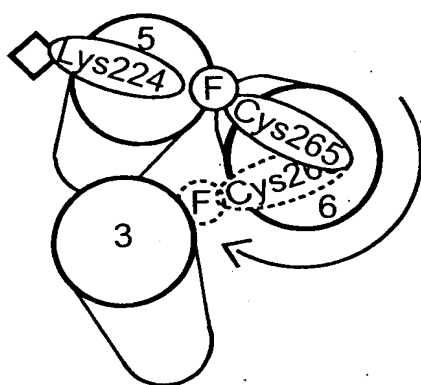


FIG. 5A

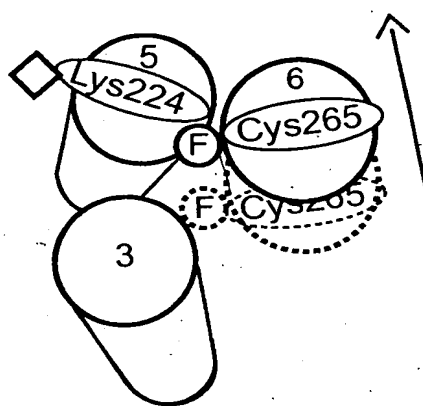


FIG. 5B

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FIG. 6A

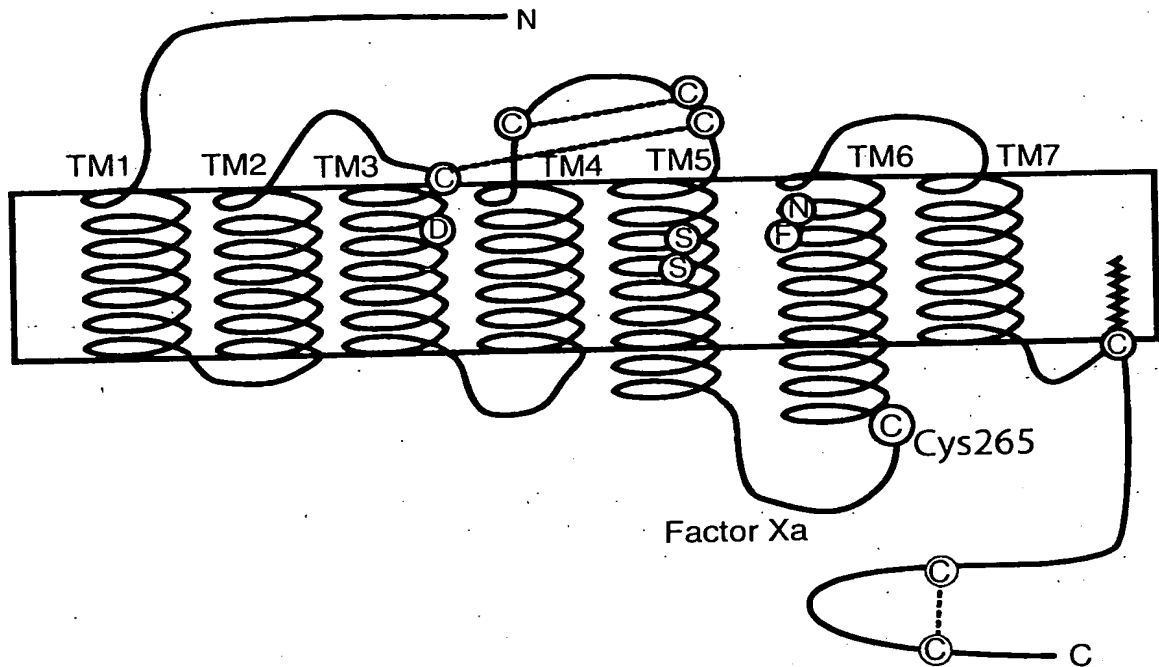
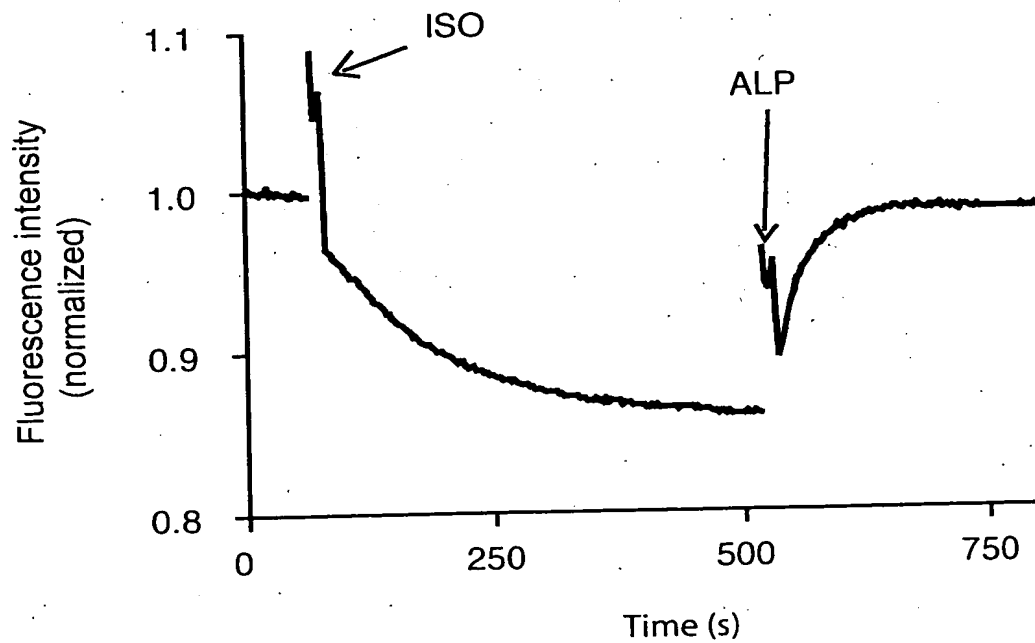
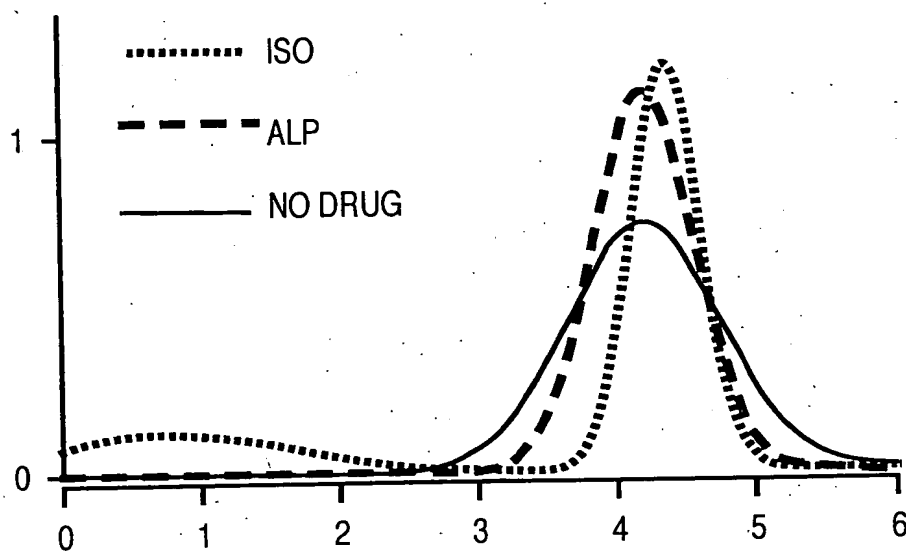


FIG. 6B



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FIG. 7



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FIG. 8A

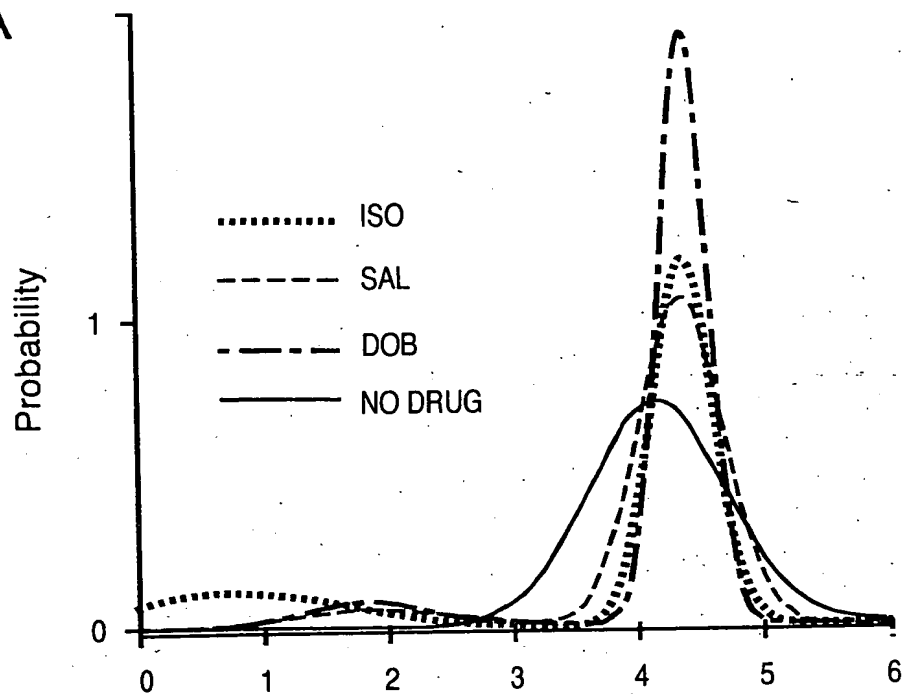
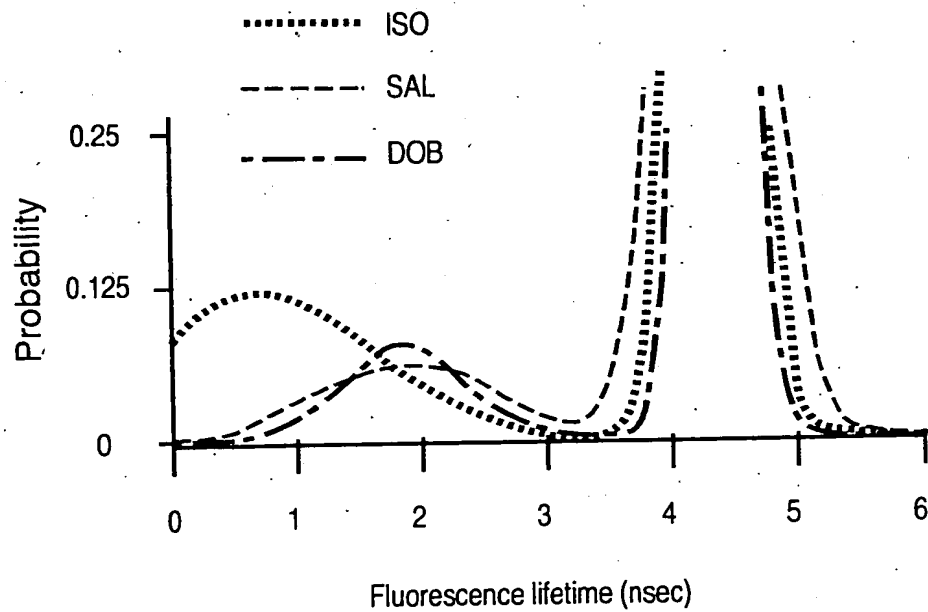


FIG. 8B



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FIG. 9A

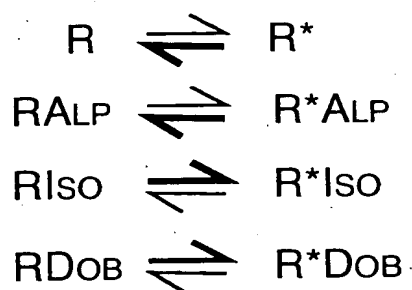
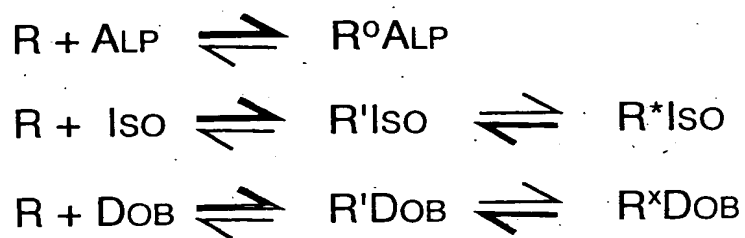
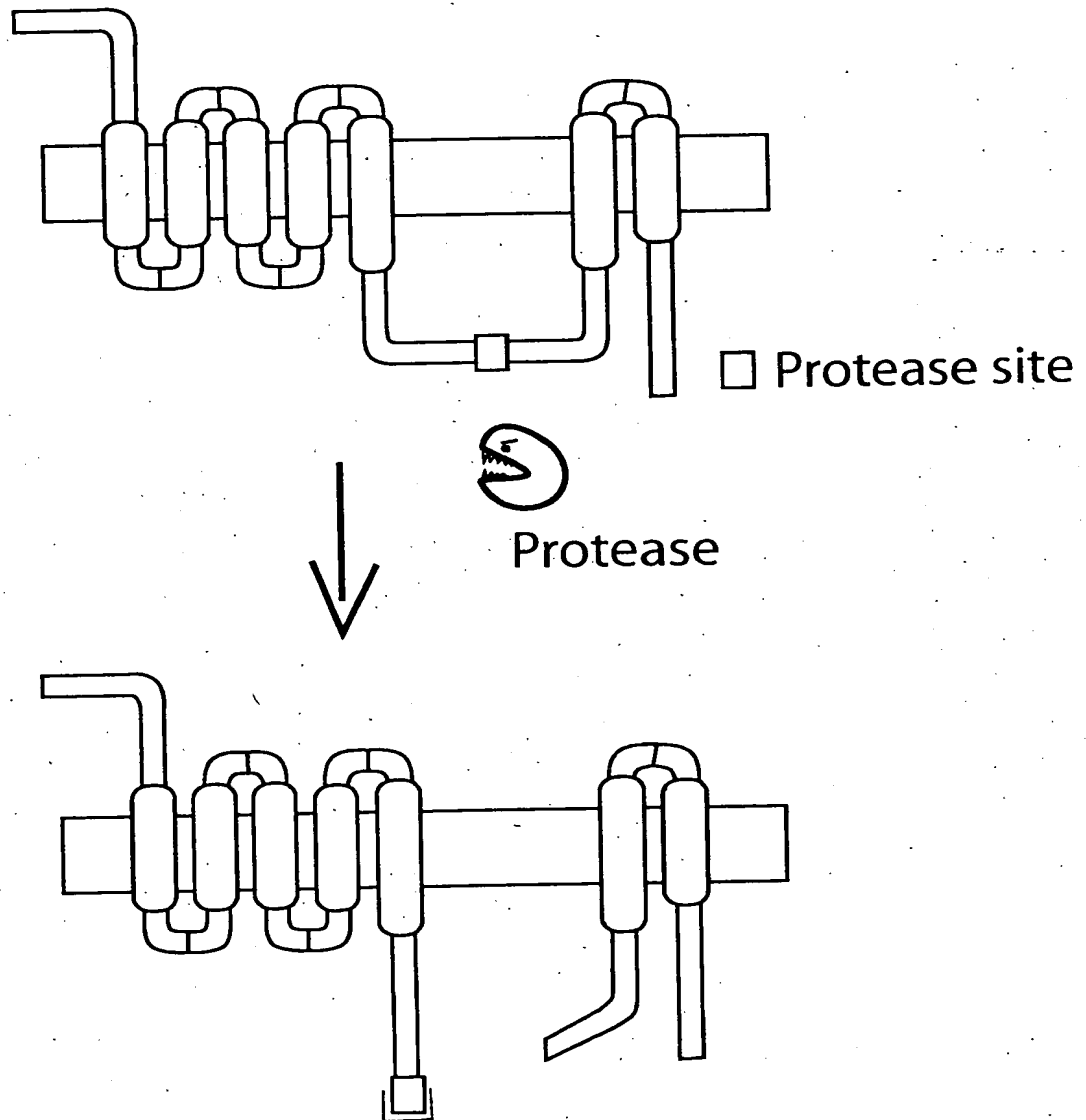


FIG. 9B



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FIG. 10



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FIG. 11A

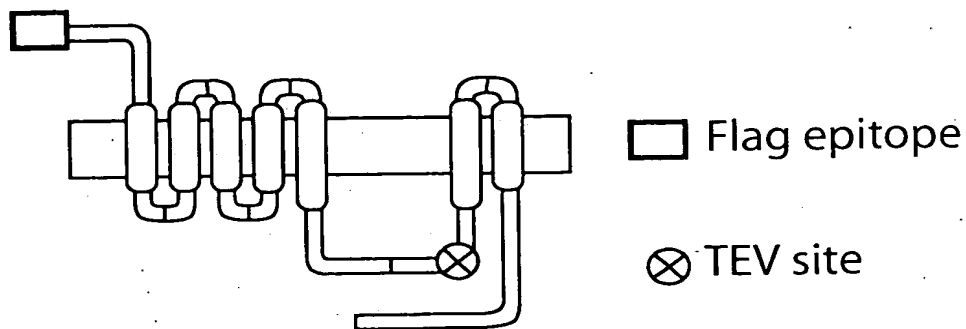


FIG. 11B

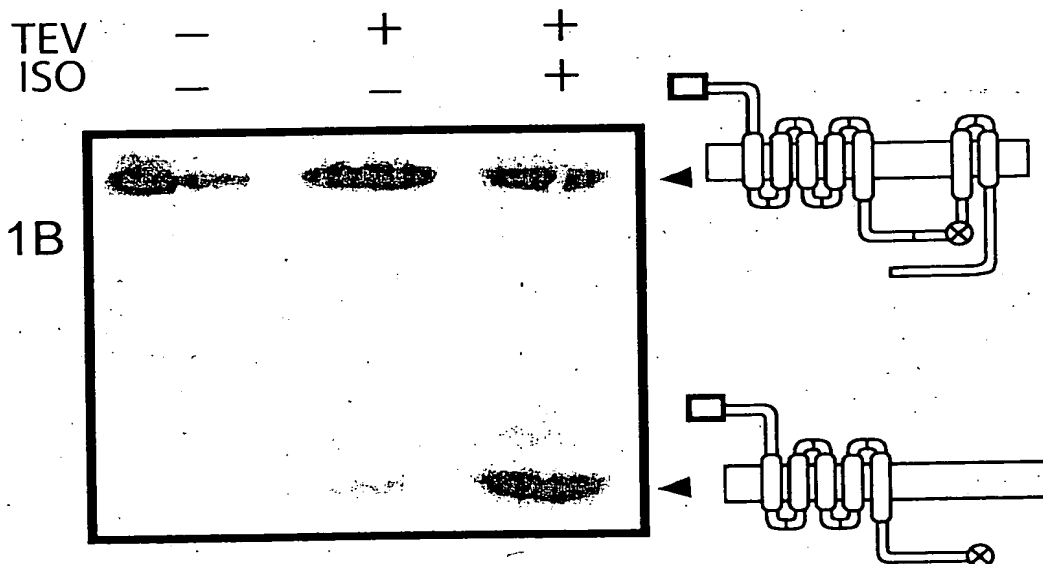
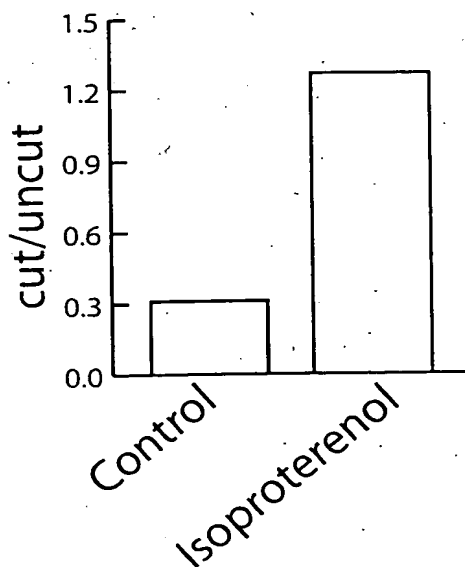


FIG. 11C



Modifications to the $\beta 2$ adrenergic receptor to add TEV protease sites

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FIG. 13
 β_2 Adrenergic Receptor DNA and Protein sequence

```
ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG CTG GCA CCC AAT AGA AGC CAT GCG CCG GAC
CAC GAC GTC CAG CAA CAG GAG GAC GAG GTG TGG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
ATC GTC CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
CTG CAG ACG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
GCA GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
TGC GAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
ATC GCA GTG GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT
AAT AAG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
ATT CAG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
I Q TGT GAC TTC TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
TGC CTG GTG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
CCC CTG GTG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
P L V GAC AAA TCT GAG E CTC GGC CAT GGA CTC GGC CAT GGA CTC GGC CAT GGA CTC
AAG ATT GAC AAA TCT GAG E CTC GGC CAT GGA CTC GGC CAT GGA CTC GGC CAT GGA CTC
CGG ACG GGC CAT GGA CTC GGC CAT GGA CTC GGC CAT GGA CTC GGC CAT GGA CTC GGC
ACG TTA GGC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC
GTG CAT GTG ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC ATC
TAT GTC AAT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT
CAG GAG CTC CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG
GGC AAC ACA GGC GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG GAG
GAA GAC CTC CCA GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
EAT TCA CAA GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
D
```

FIG. 14

β_2 Adrenergic Receptor with TEV site in 2nd intracellular loop

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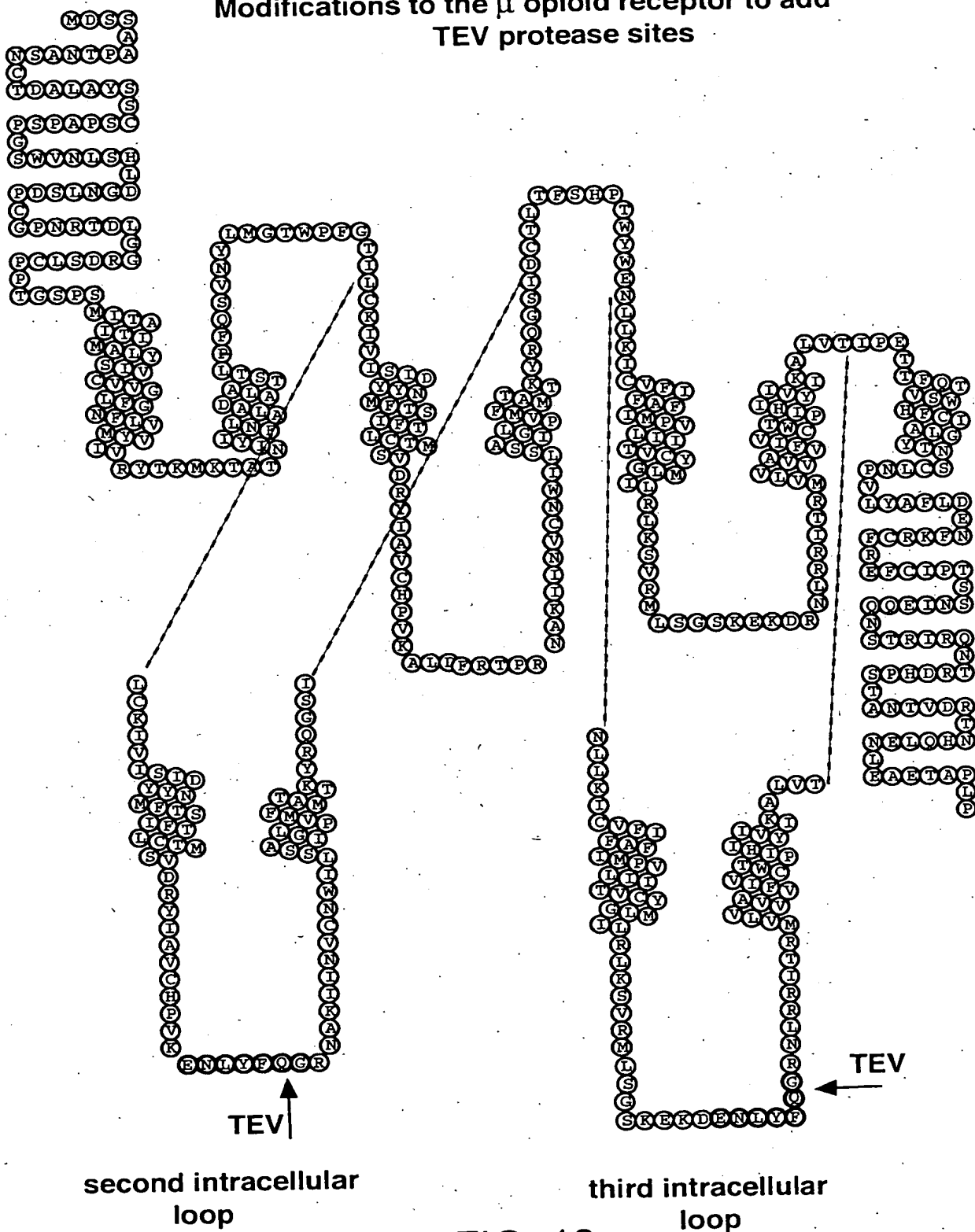
FIG. 15

b₂ Adrenergic Receptor with TEV site in 3rd intracellular loop

ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG CTG GCA CCC AAT AGA AGC CAT GCG CCG GAC
M G G CAG GTC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
CAC GAC GTC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
H D V V T T GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
ATC GTC CCG GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
I V L A ACC AAC TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG
CTG CAG ACG GTC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
L Q T T TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
GCA GTG GTG CCG GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
A V V V TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG GTG GTG GTG
TGC GAG TTT TGG ACT TCC ATT GAT GTG CCG GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG
C E F V T T TCC ATT GAT GTG CCG GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG
ATC GCA GTG GAT CGC TAC TTT TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG
I A V V R Y A T TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG
N K A A TGG TAC TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG GTG GTG
ATT CAG ATG CAC TGG TAC TTT GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG GTG
I Q M G TTT TCC ATT GAT GTG CCG GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG GTG
C C TGT GAC TTT TCC ATT GAT GTG CCG GGC CCG GGC CAA AGG GAC GAG GAG GTG GTG GTG
CCC CTG GTG ATC AAA TCT GAG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
P L V GAC AAA TCT GAG GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC GGC
K I D AAT CTC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC
R E N GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
ACG TTA GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
T L G GAT AAC GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
V H V V TTT GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
TAT GTC AAT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT
Y V N S GTC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
CAG GAG CTC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
O E L L GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
GGC AAC ACA GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
G N T T GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
GAA GAC CTC CCA GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
E D L L P G GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG
GAT TCA CAA GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG GGC CAG

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Modifications to the μ opioid receptor to add TEV protease sites



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μ Opioid receptor DNA and Protein sequence

```
ATG GAC AGC AGC AGC GCT GCC CCC ACG AAC GCC AGC AAT TGC ACT GAT GCC TTG GCG TAC TCA AGT
M   D   S   S   S   A   A   A   S   N   C   T   D   A   L   A   G   A   G   T   T   G   A   G   S
TGC TCC CCA GCA GCA TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC TCC
C   S   P   A   P   A   P   S   W   V   N   N   L   L   L   L   L   L   L   L   L   L   L   L   L   L
GAC CCA TGC GGT CCG AAC S   C   G   G   G   G   G   G   G   G   G   G   G   G   G   G   G   G   G
D   P   C   G   G   T   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C
AGT CCC TCC ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG ATG
S   P   S   M   I   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T   T
CTC TTC GGA AAC TTT CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC
L   F   N   N   F   L   N   N   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L
AAC ATC TAC ATT TTT AAC TTT AAC TTT AAC TTT AAC TTT AAC TTT AAC TTT AAC TTT AAC TTT
N   I   Y   I   F   N   N   F   L   N   N   L   L   L   L   L   L   L   L   L   L   L   L
AGT GTG AAT TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC TAC
S   V   N   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y
ATA GAT TAC TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT TAT
I   D   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y   Y
ATT GCA GTC TGC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC CAC
I   A   V   C   N   N   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C
AAT GTC TGC AAC TGG ATC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC CTC
N   V   C   N   N   W   G   G   T   T   C   S   I   L   A   T   A   T   A   T   A   T   A   T   A   T
AAA TAC AGG CAA Q   K   N   G   G   T   T   C   S   I   L   A   T   A   T   A   T   A   T   A   T
AAC CTG CTG AAG K   L   L   K   I   C   T   G   T   T   C   S   I   L   A   T   A   T   A   T
TGC TAT GGA CTG K   L   L   K   I   C   T   G   T   T   C   S   I   L   A   T   A   T   A   T
C   Y   G   A   A   T   C   T   T   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L
GAC AGG AAT CTT L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L
D   R   N   N   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L   L
TGG ACT CCC ATT CAC ATT CAC ATT CAC ATT CAC ATT CAC ATT CAC ATT CAC ATT CAC ATT
W   T   P   I   H   H   H   H   H   H   H   H   H   H   H   H   H   H   H   H   H   H
CAG ACT GTT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT TCT
Q   T   V   S   W   W   W   W   W   W   W   W   W   W   W   W   W   W   W   W   W   W
CTT TAT GCA TTT F   D   D   D   D   D   D   D   D   D   D   D   D   D   D   D   D   D
L   Y   A   F   L   C   A   A   A   A   A   A   A   A   A   A   A   A   A   A   A   A
TCC AAC ATT GAG E   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q
GCC AAT ACA GTG GAT D   R   R   R   R   R   R   R   R   R   R   R   R   R   R   R   R   R
```

FIG. 17

[illegible]

FIG. 18

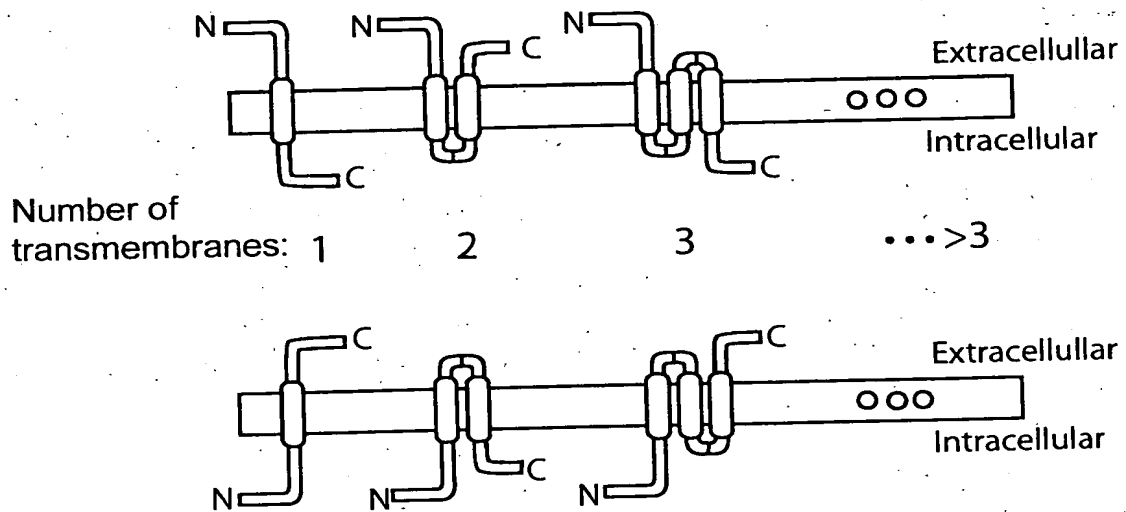
ATG	GAC	AGC	AGC	AGC	GCT	GCC	CCC	ACG	AAC	GCC	AGC	AAT	TGC	ACT	GAT	GCC	TTG	CGC	TAC	TCA	AGT
M	D	S	S	S	A	A	P	T	N	A	S	N	C	T	D	A	L	A	Y	S	S
TGC	TCC	CCA	GCA	CCA	CCC	AGC	CCC	GGT	TCC	TGG	GTC	AAC	TTG	TCC	CAC	TTA	GAT	GGC	GAC	CTG	TCC
C	S	P	A	P	P	S	P	G	S	W	V	N	L	S	H	L	D	G	D	L	G
GAC	CCA	TGC	GGT	GGT	CCG	AAC	CGC	ACC	GAC	CTG	GGC	AGA	GAC	GAC	AGC	CTG	TGC	CCT	CCA	ACC	GGC
D	P	C	G	P	P	N	R	T	D	L	G	G	R	D	S	L	C	P	P	T	G
AGT	CCC	TCC	ATG	ATC	ATC	ACG	GCC	ATC	ACG	ATC	ATG	GCC	CTC	TAC	TCC	ATC	GTG	TGC	GTG	GTG	GGG
S	P	S	M	I	I	T	A	I	T	I	M	A	L	Y	S	I	V	C	V	V	G
CTC	TTT	GGA	AAC	TTT	CTG	CTG	GTC	ATT	GTG	ATT	GTC	AGA	TAC	TAC	ACC	AAG	ATG	AAG	ACT	GCC	ACC
L	F	G	N	F	F	L	V	M	Y	V	I	V	R	Y	T	K	M	K	T	A	T
AAC	ATC	TAC	ATT	TTT	AAC	CTT	GCT	GCT	CTG	GCA	GAT	GCC	TTA	GCC	ACC	AGT	ACC	CTG	CTC	TTT	CAG
N	I	Y	I	A	T	L	A	A	L	A	D	A	L	A	T	T	S	L	P	F	Q
AGT	GTG	AAT	TAC	CTA	ATG	GGA	G	ACA	TGG	CCA	TTT	GGA	ACC	ATC	CTT	TGC	AAG	ATA	GTG	ATC	TCC
S	V	N	L	L	M	N	L	M	W	A	TTC	ACC	CTC	TGC	ACC	ATG	AGT	ATA	GAT	ATC	TAC
ATA	GAT	TAC	TAT	AAC	ATG	ATG	TTT	ACC	AGC	ATA	TTC	ACC	CTC	TGC	ACC	ATG	AGT	GTG	GAT	CGA	TAT
I	D	Y	Y	Y	N	M	F	T	S	I	F	D	L	C	T	M	S	V	D	R	Y
ATT	GCA	GTC	TGC	CAC	CCT	P	V	K	A	L	GAT	TTT	CGT	CTT	CTT	CGA	AAT	GCC	AAA	ATT	ATC
I	A	V	C	C	T	ATC	L	S	TCA	GCC	ATT	GGT	CTT	CTT	CTT	ATG	TTC	ATA	GCT	ACA	ACA
AAT	GTC	TGC	AAC	TGG	ATC	ATC	CTC	TCT	TCA	ACA	CTA	ACA	TTC	TCT	CAV	CCA	ACC	TGG	TAC	TGG	GAA
N	V	C	N	N	W	I	I	D	GAT	TGT	ACA	CTA	TTC	TCT	CAV	CCA	ACC	TGG	TAC	TGG	GAA
AAA	TAC	AGG	CAA	GGT	TCC	ATC	ATA	S	TGT	ACA	CTA	ACA	TTC	TCT	CAV	CCA	ACC	TGG	TAC	TGG	GAA
K	Y	R	Q	G	G	S	I	D	C	T	L	T	F	S	H	P	T	W	Y	W	E
AAC	CTG	CTG	AAG	ATC	TGT	ATC	TTT	TTC	ATC	TTC	GCC	ATT	ATG	ATG	CCA	GTG	CTC	ATC	ATT	ACC	GTG
N	L	L	K	I	C	V	V	F	I	F	A	F	I	M	P	V	L	I	I	T	V
TGC	TAT	GGA	CTG	ATG	ATC	TTG	TTG	CGC	CTC	AAG	AGT	GTC	CGC	ATG	CTC	TCT	GGC	TCC	AAA	GAA	AAG
C	Y	G	L	M	I	L	L	R	L	K	S	V	R	M	L	AGG	ATG	GTG	CTG	GTG	GTG
GAC	GAA	AAC	CTC	TAC	TTT	CTC	GAG	GGG	AGG	AAT	CTT	CGA	AGG	ATC	ACC	AGG	ATG	GTG	CTG	GTG	GTG
D	E	N	L	Y	Q	F	Q	G	R	N	L	R	R	I	T	R	M	V	L	V	V
GTG	GCT	GTG	ATC	ATC	ATC	GTG	TGC	TGG	ACT	CCC	ATT	CAC	ATT	TAC	GTG	ATC	ATT	AAA	GCC	TTG	GTG
V	A	V	F	I	I	V	C	W	T	P	I	H	I	Y	V	I	I	K	A	L	V
ACA	ATC	CCA	GAA	ACT	ACT	ATC	TTT	CAG	ACT	GTT	TCT	TGG	CAC	TTT	TGC	ATT	GCT	CTA	GGT		

FIG. 19

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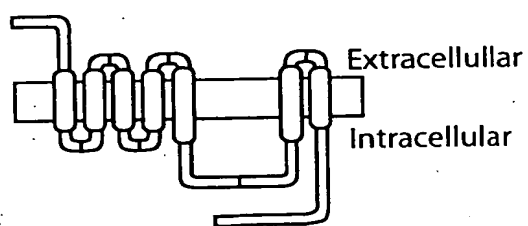
FIG. 20

Generic MSST Structure

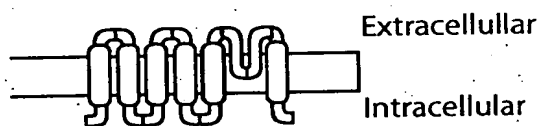


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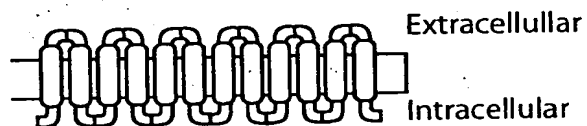
FIG. 21



GPCR



Potassium Channel



Transporter